

Amendment and Response

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Serial No.: 10/030,330

Confirmation No.: 4105

Filed: October 19, 2001

For: A POLYPEPTIDE HAVING AMIDOLYTIC ACTIVITY FOR A SERPIN

Amendments to the Claims

This listing of claims replaces all prior versions, and listings, of claims in the above-identified application:

1. **(Currently Amended)** An isolated oral bacterial polypeptide which has amidolytic activity for cleavage of a nondenatured human α_1 -proteinase inhibitor at a reactive site loop region of the inhibitor,

wherein the isolated polypeptide comprises an amino acid sequence having a percentage amino acid identity of greater than 37% to that of SEQ ID NO: 1.

2. **(Original)** The isolated polypeptide of claim 1 wherein the polypeptide has amidolytic activity in a solution comprising about 1 mM to about 500 mM Tris, about 500 μ M to about 100 mM cysteine maintained at a pH of about 7 to about 8.

3. **(Original)** The isolated polypeptide of claim 1 which is isolated from *Porphyromonas gingivalis*.

4. **(Original)** The isolated polypeptide of claim 1 which is a cysteine proteinase.

5. **(Original)** The isolated polypeptide of claim 1 which has a molecular weight of about 70 kD to about 80 kD as determined by gel filtration.

6. **(Original)** The isolated polypeptide of claim 1 which cleaves the reactive site loop region of the inhibitor represented by SEQ ID NO: 4 between glutamine and alanine.

7. **(Original)** The isolated polypeptide of claim 6 which cleaves the reactive site loop region of the inhibitor represented by SEQ ID NO: 4 between phenylalanine and leucine.

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8. **(Currently Amended)** An isolated polypeptide which is an oral bacterial cysteine proteinase and has amidolytic activity for cleavage of a nondenatured serpin at a reactive site loop region of the serpin,

wherein the isolated polypeptide comprises an amino acid sequence having a percentage amino acid identity of greater than 37% to that of SEQ ID NO: 1.

9. **(Original)** The isolated polypeptide of claim 8 wherein the polypeptide has amidolytic activity in a solution comprising about 50 mM Tris, about 20 mM cysteine maintained at a pH of about 7.4 at 37°C.

10. **(Original)** The isolated polypeptide of claim 8 which is isolated from *Porphyromonas gingivalis*.

11. **(Currently Amended)** An isolated polypeptide which is isolated from *Porphyromonas gingivalis* and has amidolytic activity for cleavage of a nondenatured serpin at a reactive site loop region of the serpin,

wherein the isolated polypeptide comprises an amino acid sequence having a percentage amino acid identity of greater than 37% to that of SEQ ID NO: 1.

12. **(Original)** The isolated polypeptide of claim 11 wherein the polypeptide has amidolytic activity in a solution comprising about 1 mM to about 500 mM Tris, about 500 μ M to about 100 mM cysteine maintained at a pH of about 7 to about 8.

13. **(Original)** The isolated polypeptide of claim 11 which has a molecular weight of about 70 kD to about 80 kD as determined by gel filtration.

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14. (Currently Amended) ~~An~~The isolated polypeptide of claim 13 ~~which is isolated from~~
Porphyromonas gingivalis and has amidolytic activity for cleavage of a nondenatured serpin at a
reactive site loop region of the serpin.

wherein the isolated polypeptide comprises ~~comprising~~ an amino acid sequence having a
percentage amino acid identity of greater than 37% to that of amino acid 148 to amino acid 843
of SEQ ID NO: 1.

15. (Original) The isolated polypeptide of claim 14 comprising an amino acid sequence having
a percentage amino acid identity of greater than 52% to that of amino acid 148 to amino acid 629
of SEQ ID NO: 1.

16. (Currently Amended) The isolated polypeptide of claim 11, wherein the polypeptide
further nonspecifically ~~which cleaves a target polypeptide nonspecifically~~ the serpin in a
denaturing environment.

17. (Canceled)

18. (Currently Amended) An isolated polypeptide comprising an amino acid sequence
represented by SEQ ID NO: 1; ~~an active analog or an active fragment thereof.~~

19. (Currently Amended) An isolated polypeptide comprising an amino acid sequence having
a percentage amino acid identity of greater than 37% to that amino acid 148 to amino acid 843 of
SEQ ID NO: 1,

wherein the polypeptide has amidolytic activity for cleavage of a nondenatured serpin at
a reactive site loop region of the serpin.

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20. **(Currently Amended)** An isolated polypeptide comprising an amino acid sequence represented by amino acid 148 to amino acid 843 of SEQ ID NO: 1, ~~an active analog or an active fragment thereof.~~

21. **(Currently Amended)** An isolated nucleic acid fragment encoding an oral bacterial polypeptide which has amidolytic activity for cleavage of a nondenatured human α_1 -proteinase inhibitor at a reactive site loop region of the inhibitor,

wherein the encoded polypeptide comprises an amino acid sequence having a percentage amino acid identity of greater than 37% to amino acid 148 to amino acid 843 of SEQ ID NO: 1,
and

wherein the nucleic acid has at least about 50% identity with SEQ ID NO:2.

22. **(Currently Amended)** An isolated nucleic acid fragment encoding a polypeptide which is an oral bacterial cysteine proteinase and has amidolytic activity for cleavage of a nondenatured serpin at a reactive site loop region of the serpin,

wherein a complement of the nucleic acid hybridizes to SEQ ID NO: 2 under hybridization conditions of 0.5 M phosphate buffer, pH 7.2, 7% SDS, 10 mM EDTA, at 68° C, followed by three 20 minute washes in 2x SSC, 0.1% SDS, at 65° C,

wherein the encoded polypeptide comprises an amino acid sequence having a percentage amino acid identity of greater than 37% to amino acid 148 to amino acid 843 of SEQ ID NO: 1,
and

wherein the nucleic acid has at least about 50% identity with SEQ ID NO:2.

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23. (Currently Amended) An isolated nucleic acid fragment encoding a polypeptide which is isolated from *Porphyromonas gingivalis* and has amidolytic activity for cleavage of a nondenatured serpin at a reactive site loop region of the serpin,

wherein a complement of the nucleic acid hybridizes to SEQ ID NO: 2 under hybridization conditions of 0.5 M phosphate buffer, pH 7.2, 7% SDS, 10 mM EDTA, at 68° C, followed by three 20 minute washes in 2x SSC, 0.1% SDS, at 65° C.

24. (Currently Amended) An The isolated nucleic acid fragment of claim 23 encoding a polypeptide which is isolated from *Porphyromonas gingivalis* and has amidolytic activity for cleavage of a nondenatured serpin at a reactive site loop region of the serpin, wherein the nucleic acid has a nucleotide sequence comprising SEQ ID NO: 2.

25. (Currently Amended) An The isolated nucleic acid fragment of claim 23 encoding a polypeptide which has amidolytic activity for cleavage of a nondenatured serpin at a reactive site loop region of the serpin,

wherein the encoded polypeptide comprises an amino acid sequence having a percentage amino acid identity of greater than 37% to amino acid 148 to amino acid 843 of SEQ ID NO: 1.

26. (Currently Amended) An The isolated nucleic acid fragment of claim 23 encoding a polypeptide which has amidolytic activity for cleavage of a nondenatured serpin at a reactive site loop region of the serpin,

wherein the encoded polypeptide comprises an amino acid sequence having a percentage amino acid identity of greater than 37% to amino acid 148 to amino acid 843 of SEQ ID NO: 1, and

wherein a complement of the nucleic acid fragment hybridizes to SEQ ID NO: 2 under hybridization conditions of 0.5 M phosphate buffer, pH 7.2, 7% SDS, 10 mM EDTA, at 68° C, followed by three 20 minute washes in 2x SSC, 0.1% SDS, at 65° C; wherein at least about 15 nucleotides of the complement hybridize.

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27. **(Currently Amended)** A method for identifying an inhibitor of a polypeptide which has amidolytic activity for cleavage of a nondenatured serpin at a reactive site loop region of a serpin comprising isolating an agent that inhibits the amidolytic activity of the polypeptide by incubating the polypeptide with the agent under conditions that promote amidolytic activity of the polypeptide and determining if the amidolytic activity of the polypeptide is reduced relative to the amidolytic activity of the polypeptide in the absence of the agent,

wherein the polypeptide comprises an amino acid sequence having a percentage amino acid identity of greater than 37% to that of SEQ ID NO: 1.

28. **(Original)** The method of claim 27 wherein the polypeptide is isolated from *Porphyromonas gingivalis*.

29. **(Currently Amended)** An immunogenic composition comprising a polypeptide which has amidolytic activity for cleavage of a nondenatured serpin at a reactive site loop region of a serpin that is capable of eliciting antibodies in an animal,

wherein the polypeptide comprises an amino acid sequence having a percentage amino acid identity of greater than 37% to that of SEQ ID NO: 1.

30. **(Canceled)**

31. **(New)** The isolated nucleic acid of claim 23 wherein the polypeptide is isolated from *Porphyromonas gingivalis*.

32. **(New)** The isolated nucleic acid of claim 25 wherein the polypeptide is isolated from *Porphyromonas gingivalis*.

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33. (New) A method of inhibiting the amidolytic activity of a polypeptide comprising combining the polypeptide with an agent selected from the group consisting of dichloroisocoumarin, diisopropylfluorophosphate, leupeptin, tosyl-L-lysine chloromethyl ketone, Phe-Pro-Arg chloromethyl ketone, Z-Phe-Lys benzoyloxy methyl ketone, idoacetamide and L-trans-epoxysuccinyl-leucylamide-(4-guanidino)-butane (E-64),

wherein the polypeptide has amidolytic activity for cleavage of a nondenatured serpin at a reactive site loop region of the serpin, and

wherein the polypeptide comprises an amino acid sequence having a percentage amino acid identity of greater than 37% to that of SEQ ID NO: 1.

34. (New) A method of inhibiting the amidolytic activity of a polypeptide comprising:

identifying an inhibitor that inhibits the amidolytic activity of the polypeptide by incubating the polypeptide with the agent under conditions that promote amidolytic activity of the polypeptide and determining if the amidolytic activity of the polypeptide is reduced relative to the amidolytic activity of the polypeptide in the absence of the agent; and

combining the polypeptide with the agent,

wherein the polypeptide has amidolytic activity for cleavage of a nondenatured serpin at a reactive site loop region of the serpin, and

wherein the polypeptide comprises an amino acid sequence having a percentage amino acid identity of greater than 37% to that of SEQ ID NO: 1.

35. (New) A kit for inhibiting the amidolytic activity of a polypeptide comprising:

an agent selected from the group consisting of dichloroisocoumarin, diisopropylfluorophosphate, leupeptin, tosyl-L-lysine chloromethyl ketone, Phe-Pro-Arg chloromethyl ketone, Z-Phe-Lys benzoyloxy methyl ketone, idoacetamide and L-trans-epoxysuccinyl-leucylamide-(4-guanidino)-butane (E-64); and

instructions for combining the agent with a polypeptide,

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wherein the polypeptide has amidolytic activity for cleavage of a nondenatured serpin at a reactive site loop region of the serpin, and

wherein the polypeptide comprises an amino acid sequence having a percentage amino acid identity of greater than 37% to that of SEQ ID NO: 1.

36. (New) An isolated nucleic acid encoding a polypeptide comprising SEQ ID NO:1.

37. (New) An isolated nucleic acid encoding a polypeptide consisting of SEQ ID NO:1.

38. (New) An isolated polypeptide comprising an amino acid sequence represented by amino acid 148 to amino acid 629 of SEQ ID NO:1.